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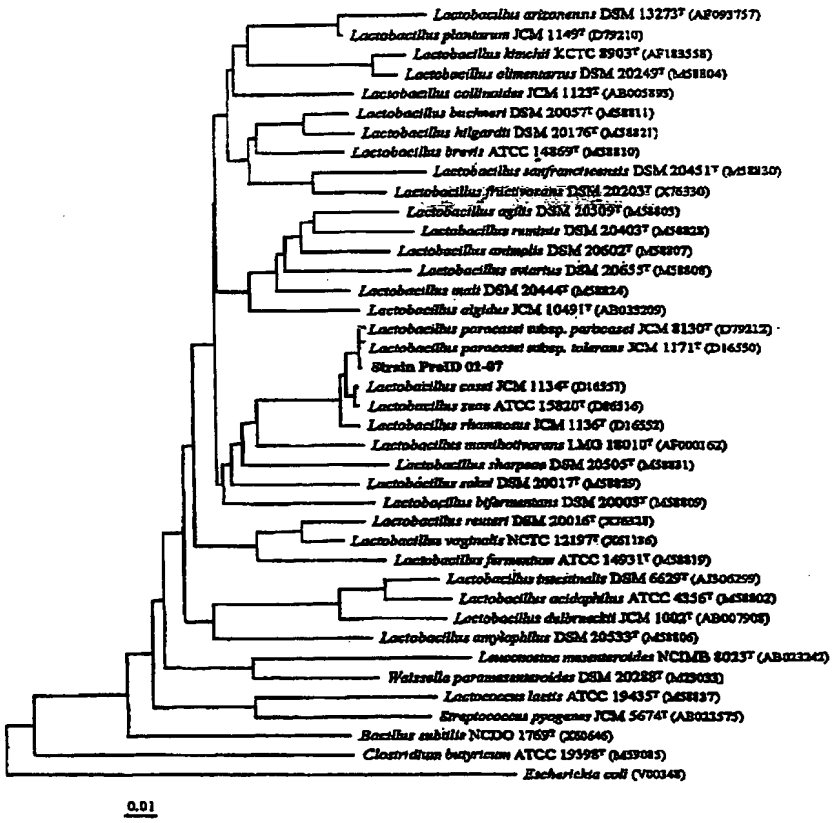

(57) Abstract: The present invention relates a novel Lactobacillus sp. Strain and use thereof. More specifically, the present invention relates to a novel Lactobacillus sp. Strain, Lactobacillus paracasei, isolated and identified from Kimchi fermentation liquid and uses thereof as probiotics, feeding additive, deodorizing agent and food additive. Lactobacillus paracasei of the present invention has good acid-resistance, bile acid-resistance and stability in the human body, has pathogenic bacteria inhibiting activity, increases numbers of beneficial bacteria by changing intestinal microflora and decreases diarrhea incidence, deodorizes offensive odor, and has effectes of softening and deodorizing in meat and fish.

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Novel Lactobacillus sp. Strain And Use Thereof

Technical Field

The present invention relates to a novel Lactobacillus sp. strain and uses thereof. More specifically, the present invention relates to a novel *Lactobacillus sp.* strain, Lactobacillus paracasei, isolated and identified from Kimchi fermentation liquid, and uses thereof as probiotics, feed additive, deodorizing agent and food additive.

Background of the Invention

Kimchi is a fermented food unique to Korea, primarily comprised of Chinese cabbage or white radish, made by adding various spices to the main vegetable ingredient and then fermenting them. Studying of microorganisms involved in the Kimchi fermentation process first began in the 1930's. Lactic acid bacteria such as the *Lactobacillus sp.*, *Pediococcus sp.* and *Leuconostoc sp.* strains, for example, are primarily representative.

Among these, *Lactobacillus sp.* strains are either homogeneous or heterogeneous fermenting *Lactic Acid Bacillus,* which can be usually found in animal and human intestinal tracts and are used in the fermentation process of dairy foods and vegetables. *Lactobacillus sp.* strains maintain intestinal pH to acidic states, thereby inhibiting the growth of harmful bacteria such as *E. coli* or Clostridium, alleviating diarrhea and constipation, functioning immunologically, as well as assisting vitamin synthesis, anti-cancer action and serum cholesterol reduction, etc. It is known that acidophillin, produced by *Lactic Acid Bacillus,* inhibits the growth of *Shigella*, *Salmonella*, *Staphylococcus*, *E. coli,* etc. Also, acidophillin inhibits the proliferation of diarrhea-causing bacteria and normalizes

intestinal microflora, thereby stopping diarrhea.

Recently, studies to develop probiotics and animal feed by using such characteristics of *Lactobacillus sp.* strains have been actively underway. The bacterial diarrhea of domestic and farm animals causes reduced growth rates and death. Thus, to prevent this condition and increase livestock productivity, adding antibiotics to animal feed has become a common practice. However, due to the advent of antibiotic-resistant bacteria and the problems associated with residual antibiotics in livestock products, policies that regulate the use of antibiotics in animal feed, and pursue organic feeding and breeding methods of animals, are gaining approval. Currently, the use of probiotics is actively recommended as an alternative for antibiotic use.

European Patent Publication No. 0861905 disclosed a novel *Lactobacillus sp.* strain, and a pharmaceutical composition and dairy product(s) for treating gastrointestinal diseases comprising the same. WO Publication No. 99/29833 disclosed Lactobacillus paracasei, a strain that is useful as probiotics and a natural drug applicable to food products. KR Patent Laid-Open Publication No. 1998-78353 disclosed a novel acid-resistant *Lactobacillus sp.* strain having harmful bacteria-inhibiting activity and farm animal probiotics comprising the same.

Summary of the Invention

The present inventors have isolated and identified a novel *Lactobacillus sp.* strain having superior acid-resistance property, bile acid-resistance property and harmful bacteria-inhibiting activity from Kimchi fermentation liquid. The present microorganism is non-toxic to humans and therefore very safe, and the said

microorganism or fermentation liquid can be used as probiotics for preventing or treating gastrointestinal-associated diseases of animals, feed additive, deodorizing agent, and food additive.

Therefore, the present invention provides a novel *Lactobacillus sp.* strain isolated from Kimchi fermentation liquid.

The present invention provides probiotics for preventing or treating animal intestinal diseases, characterized by the said *Lactobacillus sp.* strain or fermentation liquid thereof.

The present invention provides a feed additive characterized by the above said *Lactobacillus sp.* strain or fermentation liquid thereof.

The present invention provides a deodorizing agent characterized by the above said *Lactobacillus sp.* strain or fermentation liquid thereof.

The present invention provides a food additive characterized by the above said *Lactobacillus sp.* strain or fermentation liquid thereof.

Brief Description of the Figure

Figure 1 represents a phylogenic classification based on 16S rRNA sequence of Lactobacillus paracasei viro-01 of the present invention(scale bar means 0.01% substitution of the nucleotide).

Detailed description

The present invention provides a novel *Lactobacillus sp.* strain isolated from Kimchi fermentation liquid. The inventive *Lactobacillus sp.* strain is Lactobacillus paracasei viro-01, which has superior acid-resistance property, bile acid-resistance property, harmful microorganism-inhibiting activity and safety for

use in the human body.

Lactobacillus sp. strains are widely present in nature and they anaerobically metabolizes carbohydrates to produce lactic acid. Generally, it is known that when lactic acid bacteria such as *Lactobacillus sp.* strain are added directly or indirectly to food products, their metabolic product, lactic acid, improves a shelf life, flavor and texture of the food product. Also, lactic acid bacteria ingested along with fermented foods enter the intestine and adhere to intestinal epithelial cells, thereby providing beneficial effects including a pathogenic microorganism-inhibiting and antagonizing action, enhancement of immunoactivity, decrease of cancer incidence, and decrease of tumorigenic enzyme activity etc. to a host animal. Therefore, in Western and Oriental countries, lactic acid bacteria are used in processing of dairy products, meat products, pickled vegetables and various salted and pickled seafoods, as well as being used as probiotics. At this time, in order to use lactic acid bacteria as probiotics, said bacteria have to be gastric acid- or bile acid-resistant so as to be high in intestinal delivery rate, and they have to adsorb and anchor to intestinal epithelial cells or mucous membranes, and preferably secrete anti-bacterial substance to suppress harmful bacteria, thus stabilizing the intestinal environment and preventing intestinal anchoring by harmful bacteria, and they also have to be safe for use in foodstuffs. The present inventive *Lactobacillus sp.* strain, Lactobacillus paracasei viro-01 is a novel microorganism having all the above said properties.

Isolation and identification of *Lactobacillus sp.* strain of the present invention were carried out by following method. First, Kimchi fermentation liquid was serially diluted and cultivated in selection medium for lactic acid bacteria.

After isolating only lactic acid bacteria, and among these, selecting Gram positive bacillus strains, cultivation was performed in Lactobacillus-selective medium to isolate *Lactobacillus sp.* strains. Out of these, microorganisms having high acid productivity were finally isolated. Identification of said isolated

microorganisms may be carried out according to the Bergey's manual taxonomy and criteria, based on their morphological, growth and physiological properties.

For example, physiological properties such as Gram staining, oxygen-demanding property, auxotrophy, assimilation property, metabolites, enzymatic reaction or antibiotic-resistance may be used. And also, there can be used DNA sequence analysis, 16S RNA structure analysis, cell wall constituent, quinone type of electron transfer system, chemical classification methods based on fatty acid compositon of bacterial mass(MIDI) and immunological methods, for this purpose.

The present inventors analysed and identified the glucose consumption mode and the base sequence of 16S rRNA for the isolated *Lactobacillus sp.* strain and deposited the said microorganism with Korean Collection for Type Cultures, 52, Oun-dong, Yusong-ku, Taejon, Korea, an International Depository Authority under the Budapest Treaty, under Accession No. KCTC 10132BP, on Dec. 3 of 2001.

Lactobacillus paracasei viro-01(KCTC-10132BP) according to the present invention has the following characteristics.

1. This microorganism survives under the low pH stress and has strong resistance to bile acid. Specifically, Lactobacillus paracasei viro-01 of the present invention has an ability to survive under pH of less than 3.0 and 0.5% of Oxgall.

2. Lactobacillus paracasei viro-01 of the present invention has the harmful microorganism-inhibiting activity. Said harmful microorganisms include, but are not limited to, *E.coli*, *E.coli* O157, *Staphylococcus aureus, Vibrio parahaemolyticus, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus subtilis.*

3. Lactobacillus paracasei viro-01 of the present invention has a wide antibiotic-resistance. These antibiotics include, but are not limited to, Cephalexin, Flumequine, Furazolidone, Spectinomycin, Kanamycin, Gentamycin and

Neomycin.

4. Lactobacillus paracasei viro-01 of the present invention has safety in human body. Fermentation liquid of Lactobacillus paracasei viro-01 of the present invention has a unique color and flavor, has no unpleasant taste or odor, and is *E.coli* group-negative. Also, tar pigments and remaining noxious substance(Pb, Cd, Hg, As, Cr16) was not detected, thus being safe for use in the human body.

Lactobacillus paracasei viro-01 of the present invention can be cultivated in mass by conventional culturing methods of *Lactobacillus sp.* strain. As a culture medium, the medium composed of carbon source, nitrogen source, vitamins and minerals can be used, for example MRS broth(de Man- Rogosa-Sharp broth) and milk-added broth. Microorganisms can be cultivated under the conventional conditions for cultivation of lactic acid bacteria, for example, at a temperature of 15 - 45℃, for 10 - 40 hours. In order to remove media from culture and recover only concentrated bacterial mass, a centrifugation or filtration process can be performed, if desired, by those skilled in the art. Concentrated bacterial mass can be frozen or lyophilized by conventional methods to preserve it without loss of activity.

A fermentation liquid of lactic acid bacteria can be produced by using the microorganism of the present invention. To achieve this, Lactobacillus paracasei viro-01 of the present invention can be inoculated into any fruit juice and cultivated anaerobically, for example. Said fruit juice refers to liquid prepared by crushing and pressing any fruit, and there is no particular limitation to kinds of fruit juice. Example of juice which can be used include, but are not limited to, apple, orange, grape and jujube juice etc. Said fruit juice was diluted with purified water and adjusted to a brix degree of 1 to 15%. Into this diluted fruit juice was inoculated Lactobacillus paracasei viro-01 of the present invention in concentration of 1 to 5%(v/v) and incubated at 15 - 45℃, for 10 to 80 hours. pH was monitored regularly, and when it reached a range of 2.0 to 4.0, more preferably

2.8 to 3.5, the culture was transferred to an incubator at 20 to 40℃ for 5 to 10 days, during which time pH adjusted to a value of 2.0 to 5.0, preferably 3.0 to 4.0.

At that time, the fermentation was aerated for 1 hour about every 24 hours.

After completing fermentation process, fermentation liquid was recovered.

Following the recovery of fermentation liquid, one to ten volumes, more preferably one to five volumes of purified water was added to remaining lactic acid bacteria mass and precipitates in an incubator and then the incubation was continued by the same method as above. Then, purified water was added to dilute the fermentation liquid 1 to 10 times, preferably 1 to 5 times and the fermentation was aged.

Preferably, lactic acid bacteria of the present invention can be stored following concentration, centrifugation or filtration and then subsequent stabilization, lyophilization, spray drying or encapsulation of culture or fermentation liquid. For example, culture or fermentation liquid can be concentrated to remove liquid by centrifugation or filtration in the first stage, or preferably can be directly stabilized from fermentation liquid while adding filler or carrier, e.g. Aluminium silicate, diatomaceous earth, carbohydrates, sugar alcohol, starch, milk and whey powder, protein hydrolysate material, yeast and PVPP. By addition of these carriers or fillers, it is possible to obtain the solid products in a subsequent stabilization step, particularly in lyophilization, spray drying and encapsulation steps of pelletized matters.

The present invention provides probiotics for preventing or treating intestinal diseases of animals, comprising Lactobacillus paracasei viro-01 of the present invention or fermentation liquids thereof.

Term "animal" used herein, refers to mammals including a human, and preferably includes domestic and farm animal such as cattle, horse, pig etc. The term "intestinal disease" used herein includes, but is not limited to, e.g. infectious diarrhea caused by pathogenic microorganisms such as *E.coli*, Salmonella,

Clostridium, etc., gastroenteritis, inflammatory intestinal disease, neurological intestinitis syndrome, overgrowth of small intestinal microorganisms, intestinal feeding diarrhea.

Term "Probiotics" used herein, means preparation of live bacteria, i.e. microorganisms which can remain and survive in the gastrointestinal tract when they are ingested by a human or animal, and which can provide prevention or treatment of diseases. Specifically, it is a medicinal product or animal medicament made by formulating said microorganism in a live state. Generally, probiotics have an effect to treat and ameliorate the various diseases caused by abnormal growth of intestinal bacterial microflora and when administered to a human and animal, they localize and settle on the wall of the digestive tract to prevent harmful bacteria from anchoring, and produce lactic acid to thus lower the intestinal pH to prevent the proliferation of harmful bacteria. Also, probiotics produce bacteriocin and peroxides in vivo to inhibit proliferation of pathogenic bacteria and have an effect to promote action of intestinal villi responsible for nutrient absorption. In addition, they produce substances which assist the absorption and use of nutrients, improve the demanding rate of feed in livestock and also produce substances which neutralize toxic substances produced by pathogenic bacteria.

The above-said probiotics of the present invention comprise Lactobacillus paracasei viro-01 as an active ingredient and can be administered in the form of a mixture comprising said bacteria and pharmaceutically acceptable carriers by various formulations and methods.

An effective amount of Lactobacillus paracasei viro-01 means preferably more than 10^7 cells/g, more preferably, 10^7 cells/g to 10^9 cells/g. There can be used a binding agent, excipient, disintegrating agent, lubricating agent, coating agent, solubilizer, dispersant, stabilizer, suspending agent, pigment and fragrance as a carrier.

The binding agent includes, but is not limited to, polysaccharides such as

dextrin, sodium alginate, karageenan, guar gum, acacia, agar etc., naturally-occurring macromolecular substances such as tragacanth, gelatin, gluten etc., cellulosic derivatives such as hydroxypropylcellulose, methylcellulose, hydroxypropylmethylcellulose, ethylcellulose, hydroxypropylethylcellulose, sodium carboxymethylcellulose etc., and synthetic polymers such as polyvinylpyrolidone, polyvinyl alcohol, polyvinylacetate, polyethyleneglycol, polyacrylic acid, polymethacrylic acid and vinyl acetate resins.

The excipient includes sugar such as sucrose, lactose, mannitol, glucose etc. and starch such as corn starch, potato starch, rice starch, partially-pregelatinized starch etc.

The disintegrating agent includes cellulosic derivatives such as carboxymethylcellulose, calcium carboxymethylcellulose, lower-substituted hydroxypropylcellulose etc., and starch such as sodium carboxymethyl starch, hydroxypropyl starch, corn starch, potato starch, rice starch and partially-pregelatinized starch etc.

The lubricating agent includes talc, stearic acid, calcium stearate, magnesium stearate, colloidal silica, hydrous silicon dioxide, various kinds of wax and hydrogenated oil etc.

The coating agent includes water-insoluble polymers such as dimethylaminoethylmethacrylate-methacrylic acid copolymer, polyvinylacetaldiethylaminoacetate, ethylacrylate-methacrylic acid copolymer, ethylacrylate-methylmethacrylate-chlorotrimethylammoniumethylmethacrylate copolymer, ethylcellulose etc., tonic copolymer such as methacrylic acid-ethylacrylate copolymer, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetyl succinate etc., and water-soluble polymer such as methylcellulose, hydroxypropylmethylcellulose, polyvinylpyrolidone, polyethyleneglycol etc.

Probiotics of the present invention can be prepared in the form of tablet,

troche, capsule, elixir, suspension, syrup, wafer, powder, granule etc. by mixing them with the above said carriers. It is preferably administered via oral route. In addition, a pharmaceutical composition can be prepared in the various forms by conventional techniques.

Dosage can be suitably determined depending on the rate of in vivo absorption, inactivation and excretion of active ingredient, age, sex, condition of patients and severity of the disease to be treated.

The present invention provides a feed additive comprising Lactobacillus paracasei viro-01 of the present invention or fermentation liquid thereof. The feed additive of the present invention has an effect to increase useful lactic acid bacteria microflora in animal intestine, decrease the incidence of diarrhea, and increase the growth rate of the animal.

The feed additive of the present invention comprises lactic acid bacteria of the present invention or fermentation liquid thereof. Preferably, it comprises the lactic acid bacteria-fruit juice fermentation liquid prepared by inoculating the said lactic acid bacteria into fruit juice or the fermentation liquid prepared by first recovering the said lactic acid bacteria-fruit juice fermentation liquid and adding a certain amount of water to remaining bacterial mass and precipitates to dilute them, and then re-fermenting them. More preferably, it is possible to use essentially pure lactic acid bacteria culture, if desired, by adding the carrier material to said culture, and lyophilizing, spray drying, encapsulating or pelletizing it. The feed additive according to the present invention comprises the lactic acid bacteria of the present invention or the lactic acid bacteria culture prepared by the method of the present invention in amount of ranging from 10^5 to 10^{12} cells/kg, preferably 10^7 to 10^9 cells/kg.

The feed additive according to the present invention may further comprise carrier and/or filler to enhance its physiological activity. An example of the said carrier and/or filler includes, but is not limited to, aluminium silicate,

diatomaceous earth, carbohydrate, sugar alcohol, starch, milk and whey powder, protein hydrolyzate material, yeast and PVPP.

Feed additive according to the present invention can be mixed in feed during the conventional feed manufacturing process or can be mixed into animal feed in solid or liquid form when supplying feed to an animal. Said animal feed includes, but is not limited to, for example fermentation feed, compound feed, pelletized form and silage form. Fermentation feed can be prepared by adding feed additive of the present invention to organic materials and fermenting them.

Compound feed can be prepared by mixing many kinds of conventional and general feed with a feed additive of the present invention. For example, the feed additive of the present invention can be prepared by mixing more than two kinds of commercially available feed raw material in a given ratio to produce compound feed, and mixing the said compound feed with the Lactobacillus paracasei viro-01 culture of the present invention in an amount of ranging from 1 to 2 kg, based on 1 ton of compound feed. The pelletized form of said compound feed etc. can be prepared by applying heat and pressure to said compound feed in a pelletizer. Silage can be prepared by adding forage crops feed to feed additive of the present invention and fermenting them.

The present inventors confirmed that adding lactic acid bacteria fermentation liquid of the present invention to drinking water and supplying it to parent pigs and progeny pigs decreased death rate and diarrhea incidence and increased the rate of weight gain(Example 5). And also, upon determining the changes to the enteric microorganisms population in weaned piglets to which lactic acid bacteria fermentation liquid of the present invention was supplied, the number of pathogenic microorganism, Salmonella and *E.coli* decreased and the number of lactic acid bacteria significantly increased(Example 6).

Further, the present invention provides a deodorizing agent comprising Lactobacillus paracasei viro-01 or fermentation liquid thereof. The term

"Deodorizing agent" used herein means an agent capable of removing offensive odors, and in general, the deodorizing mechanism includes masking and odor-neutralization, acid-alkali neutralization, chemical mechanisms by chemical reaction, physical mechanisms by adsorbent activated charcoal, plant extracts and biological mechanisms by enzymatic degradation etc. The deodorizing agent of the present invention comprises lactic acid bacteria of the present invention or fermentation liquid thereof and thus functions to remove odor by a biological mechanism. Preferably, the deodorizing agent of the present invention comprises lactic acid bacteria-fruit juice fermentation liquid prepared by inoculating lactic acid bacteria of the present invention into fruit juice, or it comprises the fermentation liquid prepared by first recovering the said lactic acid bacteria-fruit juice fermentation liquid and then adding a certain amount of water to the remaining bacterial mass and precipitates to make them dilute and re-fermenting them. The deodorizing agent of the present invention can be provided in the form of liquid deodorizing agent consisting of the said lactic acid bacteria fermentation liquid and purified water, or in the form of powdered deodorizing agent consisting of the said liquid deodorizing agent and microorganism carrier. There can be used zeolite, activated charcoal, wollastonite, pearlite, diatomaceous earth and porous ceramic etc. as the above carriers. Preferably, deodorizing agent of the present invention can be prepared by filtering lactic acid bacteria fruit juice fermentation liquid and diluting it with purified water and adding lactic acid bacteria to a concentration ranging from about 1.0×10^5 to 1.0×10^9 cells/ml.

In order to improve palatability, deodorizing agent of the present invention can provide an odorless deodorizing agent with or without added fragrance. The fragrance includes for example, fruit aroma such as apple, papaya, lemon, orange, vanila aroma etc., flower aroma such as rose, jasmin, lilac, freesia etc., perfume such as Envy, Cooling, Perfecto, Floral, Osmanthus(brand name of perfume) etc., and plant aroma such as pinetree, cucumber, mint etc.

The deodorizing agent of the present invention can be used in the form of the spray container or can be prepared into any formulation according to intended use of the deodorizing agent. For example, the deodorizing agent of the present invention can be filled and used in spray container in a certain amount (i.e., for personal use: 90 ml, for commercial use: 500 ml) and it can be directly sprayed into the air or directly applied to an odor source.

The deodorizing agent of the present invention has a potent deodorizing effect, and thus can be applied for broad and various purposes. In other words, it is effective for deodorizing of closed narrow spaces (for example, refrigerator, bathroom), as well as for deodorizing of open spaces. The deodorizing agent of the present invention is useful for removal of offensive odors in wide and various situations, for example the removal of, but not limited to, odor of refrigerator, domestic odors of a human, animal, tobacco etc., odor of household or public toilets, odor of domestic refuse heaps or animal excretions, as well as for removal of odors of poultry farms, piggery and waste incineration yard which occasionally cause problems associated with environmental hygiene. Also, because the deodorizing agent of the present invention consists of ingredients which are not harmful to the human body, even if it comes in contact with the human body, farm animals and plants, it doesn't display toxicity to them.

Further, Lactobacillus paracasei viro-01 of the present invention or fermentation liquid thereof can be used for deodorizing of food. Therefore, the present invention provides a food composition comprising Lactobacillus paracasei viro-01 or fermentation liquid thereof. The food composition of the present invention comprises lactic acid bacteria of the present invention or fermentation liquid thereof, and preferably lactic acid bacteria fruit juice fermentation liquid prepared by inoculating fruit juice with lactic acid bacteria of the present invention, or it comprises the fermentation liquid prepared by first recovering the said lactic acid bacteria-fruit juice fermentation liquid and adding a certain amount of water

to remaining bacterial mass and precipitates to dilute them and then re-fermenting them. The food composition of the present invention can be prepared by filtering said lactic acid bacteria fruit juice fermentation liquid and then diluting it with purified water 10 to 100 times. The food composition of the present invention has softening action and an effect of decreasing offensive smells of meat and fish.

Because the said Lactobacillus paracasei viro-01 has an anti-bacterial action versus harmful pathogenic bacteria, it inhibits bacterial proliferation in food and decreases the risk of food poisoning. For example, the food composition of the present invention has an effect to decrease offensive smell and soften meat by addition of the said composition to cooked food containing meat such as steamed pig rib, panbroiled pork, broiled beef, steamed beef rib, chicken broth, fried chicken or to cooked food including fish such as broiled mackerel and spiced fish soups.

Now, the present invention was explained in detail with reference to the following examples, which are provided only for the purpose of illustrating, and are not intended to limit, the present invention.

Example 1

Isolation and identification of *Lactobacillus sp.* strain

1-1) Isolation of *Lactobacillus sp.* strain from Kimchi

Chinese cabbage Kimchi was made and fermented at 25℃ for 20 days(pH 4.0 ± 0.2) by traditional method. 1 ml of said fermented Kimchi liquid was serially diluted with sterilized physiological solution to a dilution factor of 10^1 to 10^7 and then 0.1 ml of each dilution was inoculated onto BHI(Brain Heart Infusion, Difco, USA) and incubated at 37℃ for 72 hours. Cultured bacterial mass was cultivated in BCP(Brom Cresol Purple) agar medium, a medium for detecting lactic acid whereby color changes from purple to yellow by lactic acid production,

and bacterial strains having good lactic acid production were primarily isolated.

The isolated bacterial strains were Gram stained and observed under microscope to select bacillus strains. After selecting Gram positive bacillus type microorganisms, they were incubated at 30℃ in an incubator for 48 hours using LBS(Lactobacillus-selective) medium to isolate only *Lactobacillus sp.* strains.

The number of firstly isolated strain was 30, among these we selected 10 strains, which were putative bacillus sp. strains. These bacterial strains were passaged on BHI medium.

1-2) Identification of isolated strain

The present inventors inoculated sterilized fruit juice with bacterial strains isolated from the above example 1-1, fermented them and selected the bacterial strains which were able to reduce pH of fermentation liquid below 3.0 by themselves. Selected bacterial strains were analyzed for consumption mode of substrate using API CHL Kit(Bio Merioux Corp.) according to the Manufacturer's instructions. Analysis results by API CHL Kit are shown in Table I below.

In order to allow more precise identification of the bacterial strain according to the present invention, we requested for Probionic Corp.(Korean Biotechnology Institute, Daejeon city, Republic Of Korea) to sequence the 16S rRNA gene from genomic DNA of the said strain, which was known to be diagnostic for identification of said strain.

As a result, the bacterial strain of the present invention was identified as *Lactobacillus sp.*, by molecular analysis based on 16S rRNA base sequence showing the most closely related species comprising *Lactobacillus paracasei, Lactobacillus casei, Lactobacillus zeae, Lactobacillus rhamnosus*(Figure 1).

Especially, the present bacterial strain was identified as a strain showing the closest relationship to a standard strain of Lactobacillus paracasei, with sequence homology of 99.9% in 16S rRNA. The base sequence of the inventive

bacterial strain is shown in SEQ. ID. No. 1. Consequently, the present inventors identified the bacterial strain as *Lactobacillus paracasei,* designated as *Lactobacillus paracasei* viro-01 and deposited the said microorganism with the Korean Collection for Type Cultures, 52, Oun-dong, Yusong-ku, Taejon, Korea, an International Depository Authority under the Budapest Treaty, under Accession No. KCTC 10132BP, on Dec. 3 of 2001.

Table 1

	Colony 1		Colony 2		
Contor	-	-	-	-	0
Glycerol	-	-	-	-	1
Erythritol	-	-	-	-	2
D-Arabinose	-	-	-	-	3
L-Arabinose	-	-	-	-	4
Ribose	+	+	+	-	5
D-Xylose	-	-	-	-	6
L-Xylose	-	-	-	-	7
Adonitol	+	+	+	-	8
β-Methyl-xyloside	-	-	-	-	9
Galactose	+	+	+	-	10
D-Glucose	+	+	+	-	11
D-Fructose	+	+	+	-	12
D-Mannose	+	+	+	-	13
L-Sorbose	-	-	+	-	14
Rhamnose	-	-	-	-	15
Dulsitol	-	-	-	-	16
Inositol	-	-	-	-	17
Manitol	+	+	+	-	18
Sorbitol	+	+	+	-	19
α-Methyl-D-mannoside	-	-	-	-	20
α-Methyl-D-glucoside	-	-	+	-	21
N-Acetyl glucosamine	+	+	+	-	22
Amygdalin	-	+	+	-	23
Arbutine	+	+	+	-	24

Esculine	+	+	+	-	25
Salicine	+	+	+	-	26
Cellobiose	+	+	+	-	27
Maltose	+	+	+	-	28
Lactose	-	-	-	-	29
Melibiose	-	-	-	-	30
Saccharose	-	+	-	+	31
Trehalose	+	+	+	-	32
Inuline	-	-	-	-	33
Melezitose	+	+	+	-	34
D-Raffinose	-	-	-	-	35
Amidon	-	-	-	-	36
Glycogen	-	-	-	-	37
Xylitol	-	-	-	-	38
β-Gentiobiose	-	+	+	-	39
D-Turanose	+	+	+	-	40
D-Lyxose	-	-	-	-	41
D-tagatose	+	+	+	-	42
D-Fucose	-	-	-	-	43
L-Fucose	-	-	-	-	44
D-Arabitol	-	-	-	-	45
L-Arabitol	-	+	-	+	46
Gluconate	+	+	-	+	47
2-aceto-gluconate	-	-	-	-	48
5-aceto-gluconate	-	-	-	-	49

Example 2

Characterization of the Lactobacillus paracasei viro-01 of the present invention

2-1) Acid- and bile acid-resistance test

Lactobacillus paracasei viro-01 isolated from the above example 1 was suspended in 0.85% brine with pH 2.0 and 3.0 and incubated at 37℃ hourly. The culture was diluted decimally, smeared it MRS medium and the number of colonies was counted. As a result, all the test bacterial strains were grown, and it was confirmed from this that Lactobacillus paracasei viro-01 of the present invention

possessed acid-resistance.

For the bile acid resistance test, bacterial strains were cultured in MRS liquid medium and smeared on MRS agar medium to which 0.2%, 0.3%, 0.5%, 0.7% and 1.0% of Oxgall(Difco) were added, incubated at 37℃ and then checked for growth. It was observed that all bacterial strains grew at Oxgall concentrations below 0.5%.

2-2) Determination of harmful microorganism-inhibiting activity

It was determined for harmful microorganism inhibitory activity of Lactobacillus paracasei viro-01 of the present invention. *Escherichia coli*(ATCC-25922), *Escherichia coli* O157, (ATCC-43895), *Staphylococcus aureus*(ATCC-25923), *Vibrio parahaemolyticus*(KCTC-2471), *Salmonella typhi*(KCTC-2424), and *Pseudomonas aeruginosa*(KCTC-1636) were grown in BHI liquid medium, as harmful microorganisms. For *Candida albicans*(KCTC-7965), SDB(Sabouraud Dextrose broth) was used as the medium.

After harmful microorganisms were inoculated into sterilized physiological saline solution and counted, Lactobacillus paracasei viro-01 of the present invention was added to concentration of 1%(v/v) and then left at room temperature.

After 30 sec, 2 min, 5 min and 24 hours, bacteria were counted.

As shown in Table 2, except for *Candida albicans*, significant bactericidal effects were observed. Especially, excellent effects for Vibrio sp., were observed, decreasing by 95.6% after 5 min. and completely killing all bacteria by 24 hrs.

Also, high bactericidal effects versus Salmonella typhi, *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were observed.

Table 2

Harmful microorganism-inhibiting activity of Lactobacillus paracasei of the present invention

Bacteria	Initial	30s	2min	5min	24hrs
E. coli	3.1×10^5	2.8×10^5(9.7%)	2.6×10^5(16.1%)	2.3×10^5(25.8%)	67.7%
E.coli O157	2.8×10^5	2.5×10^5(10.7%)	2.4×10^5(14.3%)	2.1×10^5(25.0%)	71.4%
Sta. aureus	2.7×10^5	2.2×10^5(18.5%)	1.4×10^5(48.1%)	1.0×10^5(63.0%)	74.1%
V.parahaem	1.1×10^5	7.5×10^4(31.8%)	2.4×10^4(78.2%)	4.8×10^3(95.6%)	100%
Sal. typhi	3.2×10^5	2.8×10^5(12.5%)	2.1×10^5(34.4%)	1.0×10^5(68.8%)	99.7%
P.aeruginos	4.8×10^5	4.6×10^5(4.2%)	4.2×10^5(12.5%)	3.8×10^5(20.8%)	70.8%
C. albicans	3.4×10^5	3.4×10^5(0.0%)	3.4×10^5(0.0%)	3.3×10^5(2.9%)	0%

2-3) Determination of bactericidal effect on Bacillus sp.

Bactericidal effect of Lactobacillus paracasei viro-01 of the present invention was measured versus Bacillus. The test strains used were *Bacillus cereus and Bacillus subtilis*, known as food poisoning bacteria. Seed cultures of the said test bacteria were smeared on nutrient agar media, incubated at 36℃ for 24 hours, and suspended in sterilized physiological saline solution. At this time, test solutions were diluted to 1.5×10^8 cfu/㎖. The test solutions were serially diluted 10^2, 10^3, 10^4 times and added, with an equal amount of the Lactobacillus paracasei viro-01 of the present invention, treated for 30 min, 1 hour, 6 hours and 24 hours, then added to nutrient agar and cultivated at 37℃ for 48 hours to observe growth. The said test solution to which the Lactobacillus paracasei viro-01 of the present invention was not added was used as a control group.

As shown in Table 3, numbers of test bacteria increased in the control group as time elapsed, but when the Lactobacillus paracasei viro-01 of the present invention was added, bacillus numbers decreased with time, and after 24 hours treated bacillus strains were completely killed. Thus, it was shown that the Lactobacillus paracasei viro-01 of the present invention had a strong bactericidal effect versus bacillus strains.

Table 3

Bactericidal effect of the Lactobacillus paracasei viro-01 of the present invention versus *Bacillus cereus and Bacillus subtilis*

treatment time	No. of Bacillus cereus(cfu/ml)			No. of Bacillus subtilis(cfu/ml)		
	10^{2*}	10^3	10^4	10^4	10^3	10^4
control	$4.8x10^2$	$9.3x10^3$	$4.3x10^4$	$2.9x10^4$	$4.8x10^3$	$8.7x10^4$
30 min. treatment	$1.4x10^2$	$4.1x10^3$	$1.2x10^4$	$1.1x10^2$	$2.3x10^3$	$9.3x10^3$
1 hr treatment	$4.6x10^2$	$2.3x10^3$	$4.9x10^2$	291.8	$1.1x10^2$	$1.0x10^2$
6 hrs treatment	-	12	13	-	9	12
24 hrs treatment	-	-	-	-	-	-

* representing dilution times of test bacterial strain

2-4) Antibiotic sensitivity

Antibiotic sensitivity of the Lactobacillus paracasei viro-01 of the present invention were tested. The antibiotic resistance test was carried out by Scientec Lab Center Co., Ltd.(Daejeon city, Daedeok, Korea) approved by the KFDA.

Antibiotics used were Cephalexin, Flumequine, Furazolidone, Gentamycin, Spectinomycin, Tetracycline, Tiamulin, Neomycin, Chloramphenicol and Kanamycin, and test procedures were as described below.

5 to 6 colonies of cultured Lactobacillus paracasei viro-01 were selected, inoculated into bacterial growth medium composed of peptone 10.0g/L, beef extract 10.0g/L, yeast extract 5.0g/L, glucose 20.0g/L, Tween 80 1.0 mL/L, K_2HPO_4 2.0g/L, sodium acetate 5.0g/L, triammonium citrate 2.0g/L, $MgSO_4.7H_2O$ 0.2g/L and $MnSO_4.4H_2O$ 0.2g/L and incubated at 37℃. Turbidity of said culture was adjusted to MacFarland No. 0.5, and used as test bacteria solution. Said test bacteria solution was smeared on Mueller Hinton agar plates and antibiotic

sensitivity test disc was added thereto and incubated at 37℃ for 16 hours.

Antibiotic sensitivity was determined by measuring the area of growth inhibition surrounding each test disc.

Experimental results showed that Lactobacillus paracasei viro-01 of the present invention had resistance to Cephalexin, Flumequine, Furazolidone, Spectinomycin, and Kanamycin. Also, the said microorganism showed moderate resistance to Gentamycin and Neomycin. On the other hand, no resistance to Tetracycline, Tiamulin and Chloramphenicol was observed(Table 4).

Table 4

Antibiotic- sensitivity test

Antibiotics	content(μg /disc)	growth-inhibition zone(mm)	resistance	Interpretation of growth-inhibiting zone(mm)		
				resistant	moderate	sensitive
sephalexin	10	0	strong	below 11	12~13	above 14
flumequine	10	0	strong	below 12	13~16	above 17
furazolidone	10	0	strong	below 11	12~13	above 14
gentamycin	10	13	moderate	below 12	13~14	above 15
spectinomycin	10	0	strong	below 11	12~13	above 14
tetracycline	30	30	none	below 14	15~18	above 19
tiamulin	10	28	none	below 11	12~13	above 14
neomycin	10	13	moderate	below 12	13~16	above 17
chloramphenicol	30	30	none	below 12	13~17	above 18
kanamycin	10	9	strong	below 13	14~17	above 18

Example 3

Production of lactic acid bacteria fruit juice fermentation liquid using Lactobacillus paracasei viro-01 of the present invention and safety test for the same

Fermentation product was prepared using the Lactobacillus paracasei viro-01 of the present invention and it was examined for safety. Said fermentation product was prepared by inoculating the Lactobacillus paracasei viro-01 of the present invention into juice of ripened fruit and fermenting to produce lactic acid bacteria fruit juice fermentation liquid. Lactobacillus paracasei viro-01 of the present invention was inoculated into MRS broth, incubated at 37℃ for 48 hours, 2% of said Lactobacillus paracasei viro-01 was inoculated into mixed fruit juice composed of sterilized apple juice 50%, orange juice 20%, grape juice 20% and jujube juice 10% and then fermented anaerobically at 30℃. Said mixed fruit juice was diluted with purified water to Brix 5 to a brix degree of 5%. After 72 hours, when pH reached 3.0, said mixed fermentation juice was fermented at 30℃ for about 7 days until it became acidity of 4.0 with aeration for 1 hour every 24 hours.

Lactic acid bacteria fruit juice fermentation liquid of the present invention was tested for *E.coli* group, number of bacteria and tar pigment, based on the general test method of Food Industry(notice of 2000-18, Korean Food & Drug Administration). As a result, said fermentation liquid was given judgement of suitability as food sauces from Korea Food & Drug Administration since it met the criteria which requires foodstuffs to have the following conditions: having unique color selectiveness and having not abnormal taste and odor, being E.coli group negative, tar pigment being not detected, and being suitable for preservative standard), and harmful remaining materials(Pb, Cd, Hg, As, Cr16) were not detected.

Example 4

Production of lactic acid bacteria fruit juice re-fermentation liquid using Lactobacillus paracasei viro-01 of the present invention.

Lactic acid bacteria fruit juice fermentation liquid of the above example 3

was recovered and remaining bacterial mass and precipitates were used for re-fermenting Lactobacillus paracasei viro-01 of the present invention. Purified water was added to the remaining bacterial mass and precipitates to dilute them 5 times, and anaerobically fermented at 30℃. After 72 hours, when pH reached 3.0, they were fermented at 30℃ for about 7 days until acidity became 4.0, while aerating for 1 hour every 24 hours. On completion of fermentation, purified water was added to the fermentation liquid to dilute it 5 times, and aged.

Example 5

Effect of Lactobacillus paracasei viro-01 of the present invention on diarrhea prevention and weight gain of parent and progeny pigs.

The present inventors investigated the effect of Lactobacillus paracasei viro-01 of the present invention on diarrhea prevention and growth of parent and progeny pigs. At a breeding farm in Ansung, Korea, 3 groups of 3 parent pigs and 30 progeny pigs were grouped and the present fermentation liquid was orally administered for 2 days to 10 days or more. At this time, to the first group was administered fermentation liquid of the present invention for 10 days and from day 10, drinking water having 0.2% of said fermentation liquid added was administered.

The second group was administered with physiological solution for 2 to 10 days or more, and from day 10, was administered with drinking water having 2% of physiological solution added. The third group was administered physiological solution for 2 to 10 days or more and from day 10, was only regular feed and water administered(Table 5). Death rate, diarrhea incidence, and weight gain for the said experimental groups were examined.

[Table 5]

Experimental group and dosage of fermentation liquid

test group	No. of parent pig	No. of progeny pig	dosage									remarks
			day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	After day 10	
1	3	30	2ml	2ml	3ml	3ml	5ml	5ml	7ml	7ml	10ml	Fermentation liquid administered (ml), 10 days later drinking water administered(0.2%)
2	3	30	2ml	2ml	3ml	3ml	5ml	5ml	7ml	7ml	10ml	physiological saline orally administered(ml), drinking water administered(0.2%)
3	3	30	2ml	2ml	3ml	3ml	5ml	5ml	7ml	7ml	10ml	physiological saline orally administered(ml), 10 days later, not administered

As an experimental result showed that upon occurrence of a premature diarrhea and a feeding diarrhea due to feed supplying to weaned piglet, administration of the present fermentation liquid decreased the death rate of animal by 6.6%, and also decreased diarrhea incidence by about 53 to 87%. Growth rate of animal also increased by 5.9%. Thus, it was confirmed that there were decrease of death rate due to diarrhea and decrease of diarrhea incidence, and effect of individual growth, when the present lactic acid bacteria fermentation liquid was administered to suckling pigs and weaned progeny pigs by means of drinking water containing 0.2% of said liquid. Also it can be seen that administration of 0.1 to 0.2% of the present lactic acid bacteria fermentation liquid-containing drinking water has contributed to improvement of productivity in farm.

Example 6

Determination of changes in intestinal flora in weaned progeny pigs to which fermentation liquid of Lactobacillus paracasei viro-01 according to the

present invention was supplied.

In order to study the changes of intestinal flora in weaned progeny pigs by supplying lactic acid bacteria fermentation liquid of the present invention, spot breeding test was performed at Chonbuk University in Korea. The test comprised establishing a control group to which the present lactic acid bacteria fermentation liquid was not supplied, a treatment group to which 0.1% of commercial antibiotic was supplied and a treatment group to which drinking water containing 0.1% of the present lactic acid bacteria fermentation liquid was supplied, respectively, and assigning 12 animals including 6 males and 6 females to each treatment group. The test period was 8 weeks.

As a result, numbers of the pathogenic microorganism, Salmonella and *E.coli* in the ileum of animals decreased in the treatment group to which the present lactic acid bacteria fermentation liquid was supplied and the treatment group to which antibiotic was supplied, compared to the control group, and numbers of beneficial bacteria increased significantly in the treatment group to which the present lactic acid bacteria fermentation liquid was supplied. Salmonella was not detected in appendix of animal known as microorganism reservoir, and *E.coli* was not present in the antibiotic treatment group and was found to be low in the treatment group to which the present lactic acid bacteria fermentation liquid was supplied, compared to control group. The number of lactic acid bacteria in appendixes of animals increased in the treatment group to which the present lactic acid bacteria fermentation liquid was supplied(Table 6)

[Table 6]

Determination of the changes in intestinal flora in weaned progeny pigs to which lactic acid bacteria fermentation liquid of the present invention was supplied.

treatment group(%)	Ileum (CFU)				Appendix(CFU)			
	Salmonella	*E.coli*	*Yeast*	*Lactobacillus*	*Salmonella*	*E.coli*	*Yeast*	*Lactobacillus*
control	2.0×10^{3}	1.0×10^{4}	2.3×10^{4}	1.2×10^{4}	0	5.0×10^{4}	3.0×10^{4}	1.1×10^{5}
antibiotic 0.1	0	3.0×10^{3}	1.8×10^{4}	1.8×10^{4}	0	0	0	4.7×10^{5}
fermentation liquid 0.1	1.0×10^{3}	6.0×10^{3}	3.0×10^{3}	2.5×10^{5}	0	2.0×10^{3}	1.0×10^{4}	6.0×10^{5}

Example 7

Test of deodorizing capacity for the deodorizing composition of the present invention

Lactic acid bacteria fermentation liquid of the present invention was diluted with purified water 150 times and investigated for the degree of removing offensive odor in a deodorizing test apparatus. As offensive odor source there were used silver methylmercaptan 50 ppm, ammonia 60 ppm, trimethylamine 60 ppm, and hydrogen sulfide 60 ppm, which were injected to the deodorizing test apparatus while controlling concentrations of the offensive odors with gastec.

Lactic acid bacteria fermentation liquid of the present invention was diluted 150 times, and 20 ml of the said liquid was sprayed through the sprayer and concentrations of odors determined after 30 min, 1 hour and 6 hours.

As a result, after 6 hours, deodorizing power versus basic odorant materials, ammonia and trimethylamine displayed removal rate of respective 85% and 80% compared to initial concentration, and after 6 hours, deodorizing power for acidic odorant materials, methylmercaptan and hydrogen sulfide displayed removal rate of respective 24% and 47% compared to initial concentration(Table 7).

[Table 7]

Deodorizing capacity of the deodorizing composition of the present invention.

odorous material	Initial	30min	1hr	6hrs
ammonia	60	19	18	9
trimethylamine	60	28	26	12
methylmercaptane	50	45	45	38
hydorgen sulfide	60	40	40	32

Example 8

Meat softening and deodorizing effect of lactic acid bacteria fermentation liquid of the present invention in meat and fish.

Reduction effect of offensive odor and meat softening effects when natural lactic acid bacteria food additive prepared from lactic acid bacteria fermentation liquid of the present invention was added to meat and fish cooking were tested.

Food additive of the present invention was lactic acid bacteria fermentation liquid of the present invention diluted with purified water 50 times. This additive was added to steamed pig rib, panbroiled pork, broiled beef, steamed beef rib, chicken broth, fried chicken, broiled mackerel, spiced fish soup and Kimchi pot stew, and the smell, toughness and taste were rated on a scale of 0 to 10. Results were statistically treated by using dispersion analysis and Duncan's Multiple range test and examined for significance. At this time, for comparison, a control material of commercially available refined rice wine was used. Amount of natural seasoning food of the present invention and refined rice wine was 2 big spoonfuls based on total of 500 g of both said meat and fish. First, meat and fish were mixed with lactic acid bacteria seasoning food or refined rice wine and then subsequent cooking steps were performed.

As a result, food additive of the present invention was superior to refined

rice wine at removing order of in meat and fish. For meat-softening action and taste, it was also confirmed that food additive of the present invention played the role of excellent natural seasonings(Table 8A-8C).

[Table 8a]

Meat softening and deodorizing effect on meat and fish

	steamed pork rib			panbroiled pork			broiled beef		
	odor	toughness	taste	odor	toughness	taste	odor	toughness	taste
1	1.650^{c}	1.9625^{b}	7.7375^{a}	1.1250^{b}	0.9375^{b}	7.7125^{a}	2.3125^{b}	2.8375^{b}	7.6250^{a}
2	2.5875^{b}	2.4125^{b}	7.4500^{a}	1.7875^{b}	1.1375^{b}	7.6000^{a}	2.3250^{b}	2.9875^{b}	7.0500^{a}
3	5.2125^{a}	5.0875^{a}	5.2250^{b}	3.4250^{a}	3.8500^{a}	5.9875^{b}	4.3375^{a}	4.7375^{c}	5.5250b
F value	81.41	59.96	28.64	16.68	82.38	39.74	23.14	10.30	14.62
Significance	***	***	***	***	***	***	***	***	***

[Table 8b]

Meat softening and deodorizing effect on meat and fish

	steamed beef rib			chicken broth			fried chicken		
	odor	toughness	taste	odor	toughness	taste	odor	toughness	taste
1	1.1875^{c}	1.8875^{c}	8.11255^{a}	1.4375^{c}	2.2250^{b}	7.7125^{a}	1.2500^{b}	2.125^{b}	8.2625^{a}
2	1.8000^{b}	2.4125^{b}	7.7625^{a}	2.1125^{b}	2.2875^{b}	7.2250^{a}	1.7000^{b}	2.2652^{b}	7.4000^{a}
3	3.0125^{a}	3.3625^{a}	6.0250^{b}	4.3875^{a}	3.7375^{a}	5.3375^{b}	3.6375^{a}	4.1375^{a}	6.1000^{b}
F value	22.37	22.92	16.62	55.16	10.87	36.40	31.74	14.98	13.29
Significance	***	***	***	***	***	***	***	***	***

[Table 8c]

Meat softening and deodorizing effect on meat and fish

	broiled mackerel			spiced fish soup		Kimchi pot stew	
	odor	hardness	taste	odor	taste	odor	taste
1	1.2125^{c}	1.5125^{a}	7.9875^{a}	1.6000^{b}	8.1500^{a}	1.8875^{b}	8.5375^{a}
2	2.2750^{b}	1.4500^{a}	7.9250^{a}	1.9250^{b}	7.8875^{a}	2.0375^{b}	7.8000^{b}
3	3.5125^{a}	1.8000^{a}	7.2000^{b}	3.3625^{a}	6.3125^{b}	4.5750^{a}	6.3125^{c}
F value	29.87	1.41	4.36	16.29	26.43	44.33	22.00
Significance	***	.	*	***	***	***	***

1) 1: food additive of the present invention added, 2: refined rice wine added, 3: control group

2) Duncan's multiple range test: comparing each sample at a given property value, thereby being considered significance($p<0.05$) if not the same alphabetic symbol.

3) significance: ***; significant ($p<0.001$), * : significant ($p<0.1$)

Applicant's or agent's File reference OP020021	International application No. PCT/KR02/00914

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 16, line 3~6

B. IDENTIFICATION OF DEPOSIT Further deposits are on an additional sheet ☐

Name of depositary institution

Korean Collection for Type Cultures

Address of depositary institution(*including postal code and country*)

#52, Oun-dong, Yusong-ku, Taejon 305-333,
Republic of Korea

Date of deposit	Accession Number
03/12/2001	KCTC 10132BP

C. ADDITIONAL INDICATIONS*(leave blank if not applicable)* This information is continued on an additional sheet ☐

D.DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E.SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later*(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Claims

1. Lactobacillus paracasei viro-01(KCTC 10132BP).

2. Fermentation liquid of Lactobacillus paracasei viro-01(KCTC 10132BP).

3. Probiotics for preventing or treating intestinal diseases of animals, comprising Lactobacillus paracasei viro-01(KCTC 10132BP) or fermentation liquid thereof.

4. The probiotics of claim 3, wherein the said intestinal disease is infectious diarrhea caused by pathogenic microorganisms, gastroenteritis, inflammatory intestinal disease, neurological intestinitis syndrome, overgrowth of small intestinal microorganisms and intestinal feeding diarrhea.

5. Feed additive comprising Lactobacillus paracasei viro-01(KCTC 10132BP) or fermentation liquid thereof.

6. Deodorizing agent comprising Lactobacillus paracasei viro-01(KCTC 10132BP) or fermentation liquid thereof.

7. Food composition comprising Lactobacillus paracasei viro-01(KCTC 10132BP) or fermentation liquid thereof.

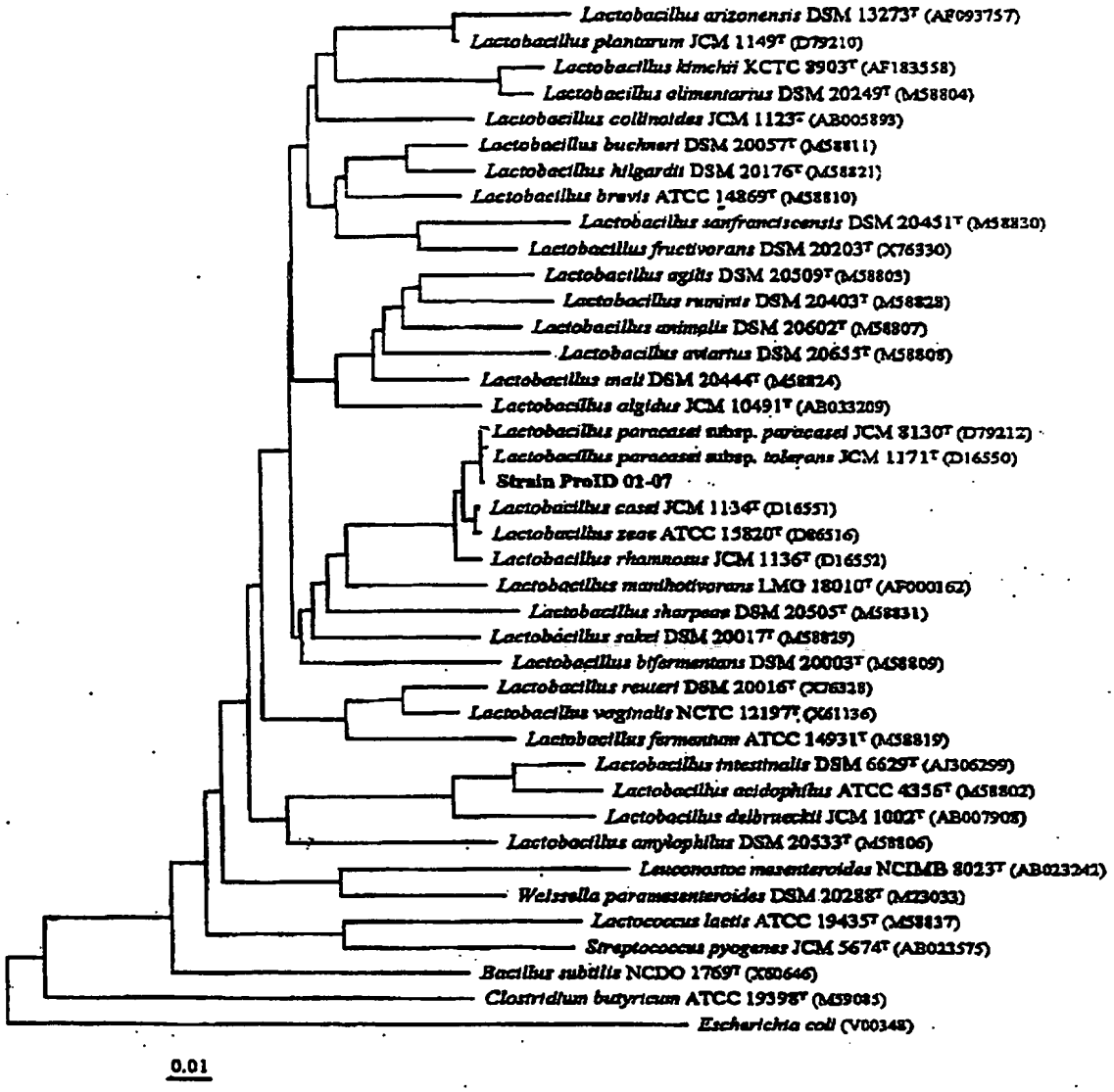
8. Method for preparing fermentation liquid of Lactobacillus paracasei viro-01(KCTC 10132BP).

9. The method of claim 8, comprising inoculating Lactobacillus paracasei

viro-01(KCTC 10132BP) into fruit juice, anaerobically fermenting them at 15 to 45℃, and then fermenting them at 20 to 40℃ with aeration until acidity reached value of 2.0 to 5.0.

10. Method of claim 8 comprising the step(s) of, after recovering the first lactic acid bacteria fermentation liquid, adding purified water to remaining lactic acid bacterial mass and precipitates to a dilution of 1 to 10 times, re-fermenting, and adding purified water to dilution of 1 to 10 times and aging.

FIG. 1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Lactobacillus collinoides JCM 1123T (AB005893)
Lactobacillus buchneri DSM 20057T (M58811)
Lactobacillus hilgardii DSM 20176T (M58821)
Lactobacillus brevis ATCC 14869T (M58810)
Lactobacillus sanfranciscensis DSM 20451T (M58830)
Lactobacillus fructivorans DSM 20203T (X76330)
Lactobacillus agilis DSM 20509T (M58803)
Lactobacillus ruminis DSM 20403T (M58828)
Lactobacillus animalis DSM 20602T (M58807)
Lactobacillus aviarius DSM 20655T (M58808)
Lactobacillus mali DSM 20444T (M58824)
Lactobacillus algidus JCM 10491T (AB033209)
Lactobacillus paracasei subsp. paracasei JCM 8130T (D79212)
Lactobacillus paracasei subsp. tolerans JCM 1171T (D16550)
Strain ProID 01-07
Lactobacillus casei JCM 1134T (D16551)
Lactobacillus zeae ATCC 15820T (D86516)
Lactobacillus rhamnosus JCM 1136T (D16552)
Lactobacillus manihotivorans LMG 18010T (AF000162)
Lactobacillus sharpeae DSM 20505T (M58831)
Lactobacillus sakei DSM 20017T (M58819)
Lactobacillus bifermentans DSM 20003T (M58809)
Lactobacillus reuteri DSM 20016T (X76328)
Lactobacillus vaginalis NCTC 12197T (X61136)
Lactobacillus fermentum ATCC 14931T (M58819)
Lactobacillus intestinalis DSM 6629T (AJ306299)
Lactobacillus acidophilus ATCC 4356T (M58802)
Lactobacillus delbrueckii JCM 1002T (AB007908)
Lactobacillus amylophilus DSM 20533T (M58806)
Leuconostoc mesenteroides NCIMB 8023T (AB023242)
Weissella paramesenteroides DSM 20288T (M23033)
Lactococcus lactis ATCC 19435T (M58837)
Streptococcus pyogenes JCM 5674T (AB023575)
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PARK, Soon Duck

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cggtatccaa ccagaaagcc acggctaact acgtgccagc agccgcggta atacgtaggt 540

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Sequence Listing

tgtgaaagcc ctcggcttaa ccgaggaagc gcatcggaaa ctgggaaact tgagtgcaga 660

agaggacagt ggaactccat gtgtagcggt gaaatgcgta gatatatgga agaacaccag 720

tggcgaaggc ggctgtctgg tctgtaactg acgctgaggc tcgaaagcat gggtagcgaa 780

caggattaga taccctggta gtccatgccg taaacgatga atgctaggtg ttggagggtt 840

tccgcccttc agtgccgcag ctaacgcatt aagcattccg cctggggagt acgaccgcaa 900

ggttgaaact caaaggaatt gacgggggcc cgcacaagcg gtggagcatg tggtttaatt 960

cgaagcaacg cgaagaagct taccaggtct tgacatcttt tgatcacccg agagatcagg 1020

tttccccttc gggggcaaaa tgacaggtgg tgcatggttg tcgtcagctc gtgtcgtgag 1080

atgttgggtt aagtcccgca acgagcgcaa cccttatgac tagttgccag catttagttg 1140

ggcactctag taagactgcc ggtgacaaac cggaggaagg tggggatgac gtcaaatcat 1200

catgcccctt atgacctggg ctacacacgt gctacaatgg atggtacaac gagttgcgag 1260

accgcgaggt caagctaatc tcttaaagcc attctcagtt cggactgtag gctgcaactc 1320

gcctacacga agtcggaatc gctagtaatc gcggatcagc acgccgcggt gaatacgttc 1380

ccgggccttg tacacaccgc ccgtcacacc atgagagttt gtaacacccg aagccggtgg 1440

cgtaaccctt ttagggagcg agccgtctaa ggtgggacaa atgattaggg tgaagtcgta 1500

acaaggtagc cgtaggagaa cctgc 1525

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00914

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 1/20, 1/00 : C12P 7/56 : A23K 1/00, 1/16, 1/165

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the internatnational search (name of data base and, where practicable, search terms used)
PubMed, Delphion, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9929833 (ARLA EKONOMISK FORENING) Jun 1999 See the claims	1, 3-12
A	KR 1020000047065 (HO-GOON PARK) Jul 2000 See the whole document	1, 3, 4
A	Martha I. Alvarez-Olmos and Richard A. Oberhelman "Probiotic Agents and Infectious Diseases: A Modern Perspective on a Traditional Therapy" Clinical Infectious Diseases vol.32:1567-1576, May 2001 See the abstract	1, 3-12
A	Gorbach SL. "Probiotics and gastrointestinal health" Am J Gastroenterol vol 95(1 Suppl):S2-4, Jan 2001 See the abstract	1, 3-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 SEPTEMBER 2002 (17.09.2002)	18 SEPTEMBER 2002 (18.09.2002)

Name and mailing address of the ISA/KR
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00914

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Due to the broad and indefinite scope, the meaningful search for claim 2 is not possible to be carried on.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00914

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929833A1	June 17, 1999	EP 1036160A1	Sep 20, 2000
		CA 2313245AA	Jun 17, 1999
		JP 20015260T2	Dec 18, 2001
		PL 341010A1	Mar 12, 2001
		NO 20002919A	Aug 8, 2000
KR 1020000047065	July 25, 2000	None	